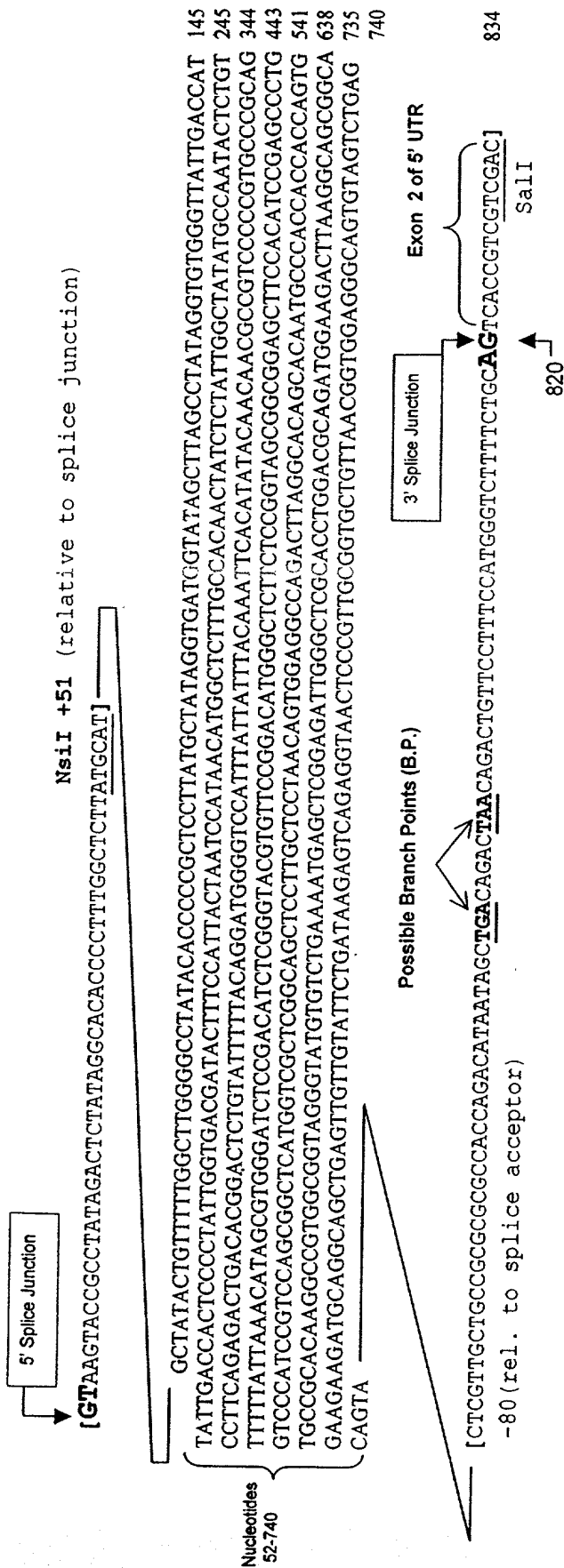


**FIG. 1A : Full length Intron A = 820 bp**



**FIG. 1B : Synthetic Oligo for substitution of nucleotides 52-740 of Intron A**

[ATGCATCTCGTTGCTGCGCGCGGCCACAGACATAATCGCTGACACACTGACAGACTGTTCCCTTTTCTTTTTCGAGTCACCGTCTCGAC]  
NsiI -80  
Changed to B.P. consensus  
Changed to polypyrimidine consensus Sali

**FIG. 1C : Deletion Mutant pCON3 Intron: 132 bp**

NsiI

Branch Point

[GTAAGTACCGCTATAGACTCTATAGGCACACCCCTTTGGCTCTTATGCATCTGCTGCTCCCGCGGCCACAGACATAATCGCTGACACACTGA  
CAGACTGTTCCCTTTCTTTTTCGAGTCACCGTCTCGAC]  
Polypyrimidine Tract Sali

1 ctgcagtgaa taataaaatg tgtgtttgtc cgaaatacgc gttttgagat ttctgtcgcg  
 61 gactaaattc atgtcgcgcg atagtgggtg ttatcgccga tagagatggc gatattggaa  
 121 aaatcgatat ttgaaaatat ggcatattga aaatgtcgcc gatgtgagtt tctgtgtaac  
 181 tgatatcgcc atttttccaa aagtgtttt tgggcatacg cgatatctgg cgatacggct  
 241 tatatcgttt acgggggatg gcgatagacg actttggcga cttgggcgat tctgtgtgtc  
 301 gcaaatatcg cagtttcgat ataggtgaca gacgatatga ggctatatcg ccgatagagg  
 361 cgacatcaag ctggcacatg gccaatgcat atcgatctat acattgaatc aatattggca  
 421 attagccata ttagtcattg gttatatagc ataaatcaat attggctatt ggccattgca  
 481 tacgtttgat ctatatcata atatgtacat ttatattggc tcatgtccaa tatgaccgcc  
 541 atgttgacat tgattattga ctagtattta atagtaatca attacggggg cattagttca  
 601 tagcccatat atggagttcc gcgttacata acttacggta aatggcccgc ctctgacccg  
 661 cccaacgacc cccgcccatt gacgtcaata atgacgtatg ttcccatagt aacgccaata  
 721 gggactttcc attgacgtca atgggtggag tatttacggg aaactgccc a ttggcagta  
 781 catcaagtgt atcatatgcc aagtcgggcc cctattgac gtcaatgacg gtaaatggcc  
 841 cgcctggcat tatgcccagt acatgacctt acgggacttt cctacttggc agtacatcta  
 901 cgtattagtc atcgctatta ccatggtgat gcggttttgg cagtacacca atgggagctg  
 961 atagcggttt gactcacggg gatttccaag tctccacccc attgacgtca atgggagttt  
 1021 gttttggcac caaaatcaac gggactttcc aaaatgtcgt aataaccccg ccccggtgac  
 1081 g<sup>+</sup>caaatgggc ggtaggcgtg tacggtggga ggtcta<sup>+</sup>tata agcagagctc gtttagtgaa  
 1141 ccg<sup>+</sup>tcagatc gcctggagac gccatccacg ctgttttgac ctccatagaa gacaccggga  
 1201 ccgatccagc ctccgcggcc gggaacgggtg cattggaacg cggattcccc gtgccaagag  
 1261 tgacGTAAGT ACCGCCTATA GACTCTATAG GCACACCCCT TTGGCTCTTA TGCATGCTAT  
 1321 ACTGTTTTTG GCTTGGGGCC TATACACCCC CGCTCCTTAT GCTATAGGTG ATGGTATAGC  
 1381 TTAGCCTATA GGTGTGGGTT ATTGACCATT ATTGACCACT CCCCTATTGG TGACGATACT  
 1441 TTCCATTACT AATCCATAAC ATGGCTCTTT GCCACAAC TA TCTCTATTGG CTATATGCCA  
 1501 ATACTCTGTC CTTCAGAGAC TGACACGGAC TCTGTATTTT TACAGGATGG GGTCCCATT  
 1561 ATTATTTACA AATTCACATA TACAACAACG CCGTCCCCCG TGCCCGCAGT TTTTATTAAA  
 1621 CATAGCGTGG GATCTCCACG CGAATCTCGG GTACGTGTTC CGGACATGGG CTCTTCTCCG  
 1681 GTAGCGGCGG AGCTTCCACA TCCGAGCCCT GGTCCCATGC CTCCAGCGGC TCATGGTCGC  
 1741 TCGGCAGCTC CTGCTCCTA ACAGTGGAGG CCAGACTTAG GCACAGCACA ATGCCACCA  
 1801 CCACCACTGT GCCGCACAAG GCCGTGGCGG TAGGGTATGT GTCTGAAAAT GAGCTCGGAG  
 1861 ATTGGGCTCG CACCGTGACG CAGATGGAAG ACTTAAGGCA GCGGCAGAAG AAGATGCAGG  
 1921 CAGCTGAGTT GTTGATTCT GATAAGAGTC AGAGGTAAC CCCGTTGCGG TGCTGTTAAC  
 1981 GGTGGAGGGC AGTGTAGTCT GAGCAGTACT CGTTGCTGCC GCGCGGCCA CCAGACATAA  
 2041 TAGCTGACAG ACTAACAGAC TGTTCTTTT CATGGGTCTT TTCTGCAGtc accgtccttg  
 2101 acacgatgga gtcctctgcc aagagaaag<sup>+</sup>tgaccctga taatcctgac gagggccctt  
 2161 cctccaaggt

Enhancer Region  
 (~600 - ~1081)

Pol II Promoter  
 (1081 - 1143)

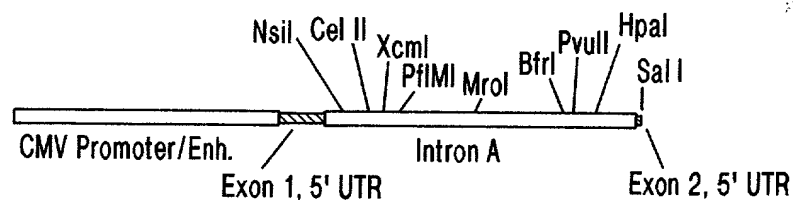
Exon 1 (5' UTR)  
 (1144 - 1264)

Intron A  
 (1265 - 2088)

Exon 2 (5' UTR,  
 Start of Trl.)  
 (2089 - )

FIG. 2

# Deletions Made Within Intron A of CMV IE1



DELETION	LENGTH*		
Nsi-Cel II	70 bp		Nsi - X Truncations
Nsi-XcmI	113 bp		
Nsi-PflMI	150 bp		
Nsi-MroI	345 bp		
Nsi-BfrI	578 bp		
Nsi-Pvull	609 bp		
Nsi-HpaI	663 bp		

DELETION	LENGTH*		
Hpa-Pvull	54 bp		Hpa - X Truncations
Hpa-BfrI	80 bp		
Hpa-MroI	314 bp		
Hpa-PflMI	516 bp		
Hpa-Cel II	590 bp		

\* Following restriction enzyme digestion, building, religation

FIG. 3

# Intron A Internal Deletion Mutants (Transiently-Transfected 293 cells)

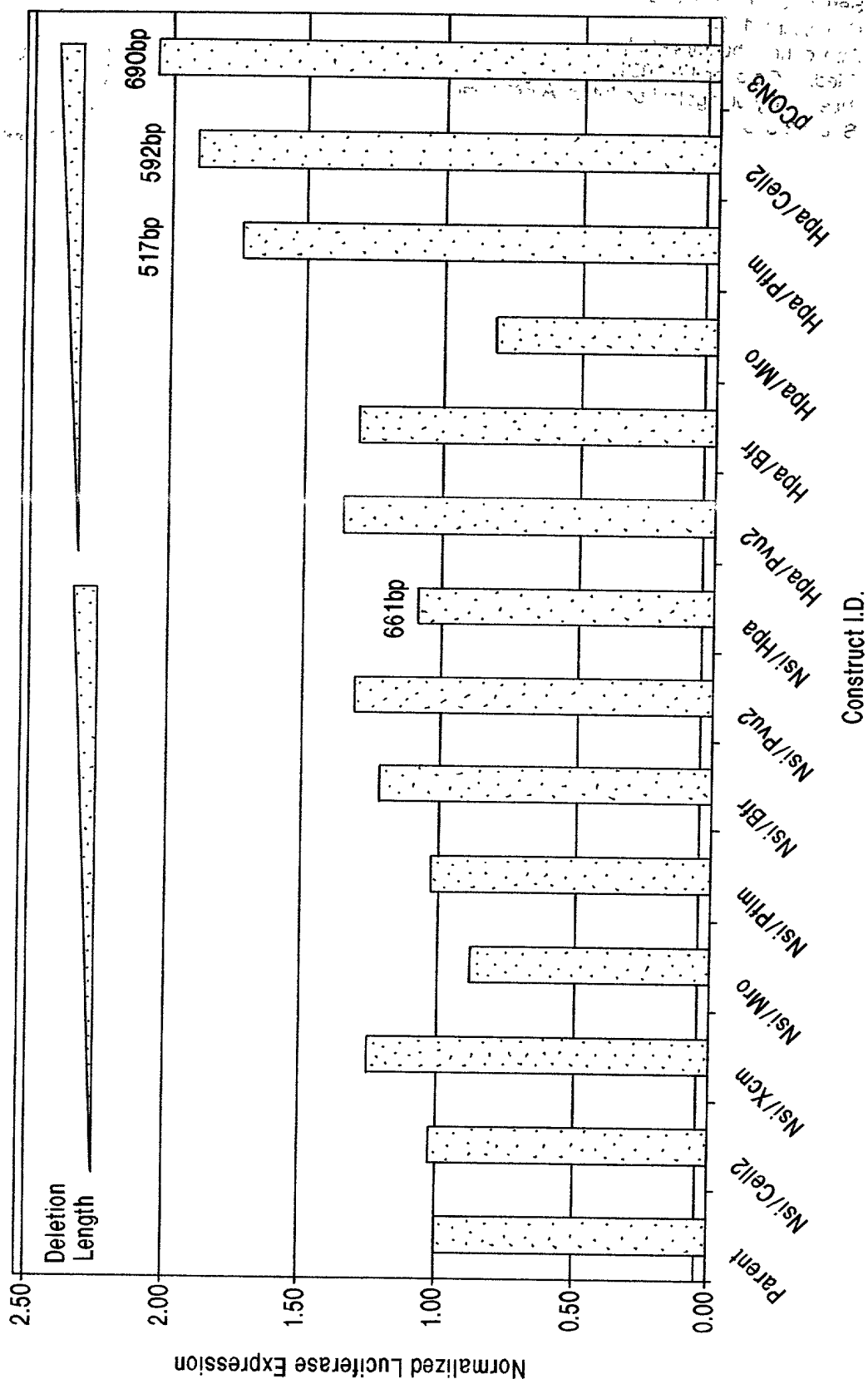


FIG. 4

1. Wild Type Rabbit  $\beta$ -Globin Sequence

GTTGGTATCCTTTTACAGCACAACTTAATGAGACAGATAGAAACTGGTCTTGTAGAAACA  
Splice Donor

GAGTAGTCGCCTGCTTTTCTGCCAGGTCTGACTTCTCTCCCCTGGGCTGTTTTCATTTTCTCAG  
Branch Pt. Polypyrimidine Tract

FIG. 5A

2. Optimized Rabbit  $\beta$ -Globin Sequence

GTAAGTATCCTTTTACAGCACAACTTAATGAGACAGATAGAAACTGGTCTTGTAGAAACA  
Splice Donor

GAGTAGTCGCCTGCTTTTCTGCCAGGTACTAACTTCTCTCCCCTCTCCTCTTTTCTTTTCTGCAG  
Branch Pt. Polypyrimidine Tract

FIG. 5B

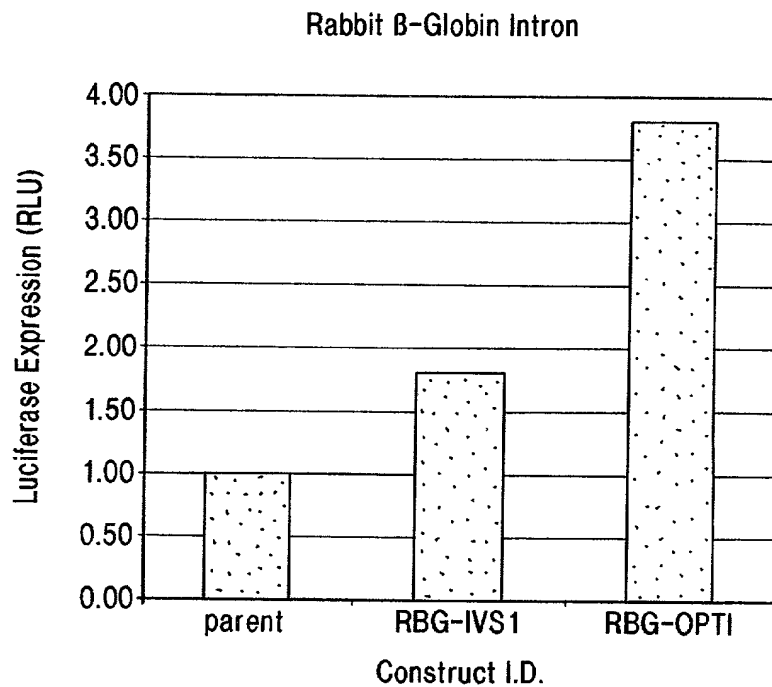
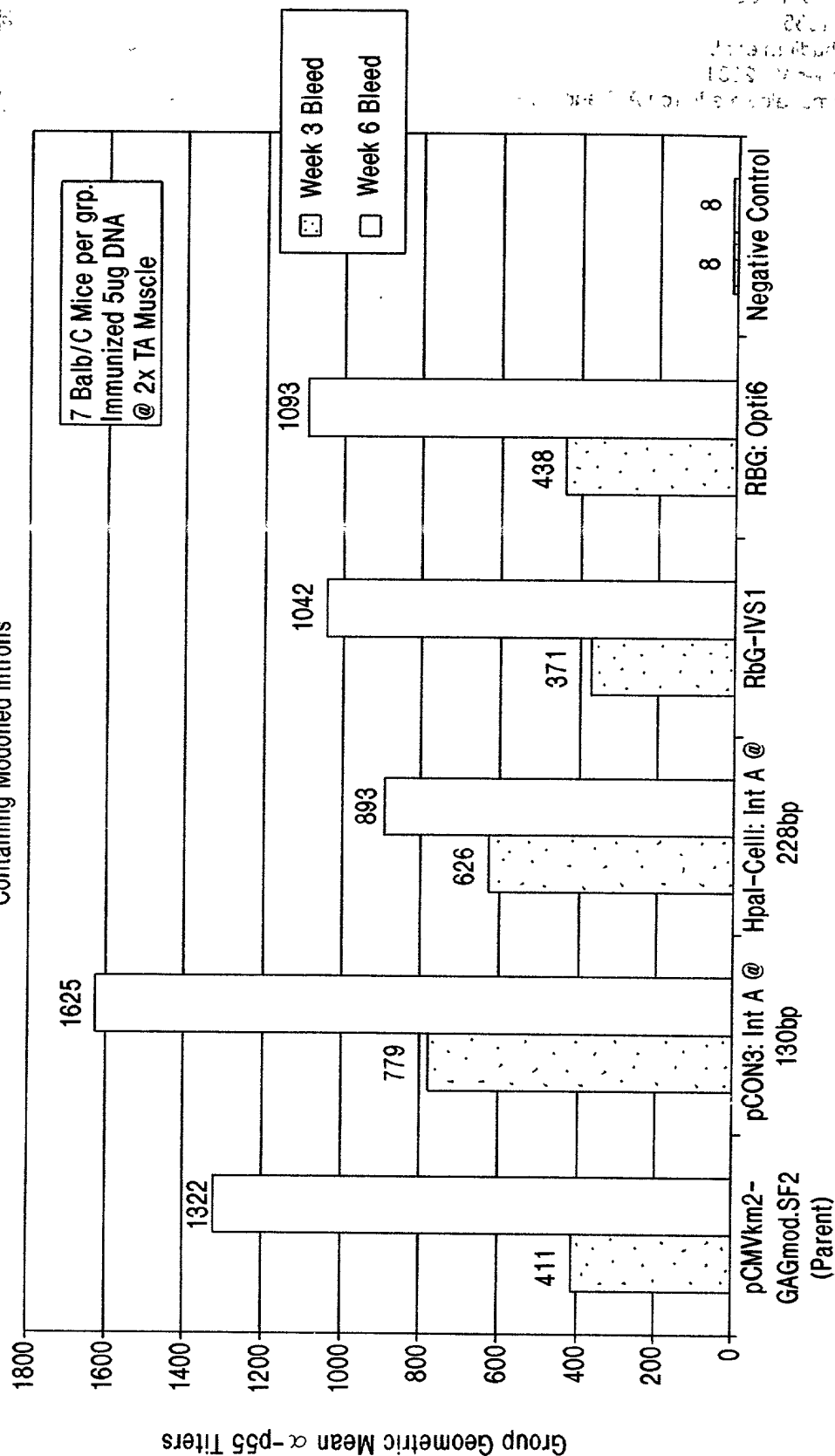


FIG. 6

# In Vivo Immunogenicity of Plasmid Vectors Containing Modified Introns



Immunizing Vector DNA

FIG. 7

FIG. 7 is a bar chart showing the group geometric mean α-p55 titers for various immunizing vector DNAs at Week 3 and Week 6. The y-axis represents the Group Geometric Mean α-p55 Titers, ranging from 0 to 1800. The x-axis lists the immunizing vector DNAs: pCMV/km2-GAGmod.SF2 (Parent), pCON3: Int A @ HpaI-Cell: Int A @ 130bp, HpaI-Cell: Int A @ 228bp, RbG-IVS1, RbG: Opti6, and Negative Control. For each vector, two bars are shown: a solid bar for Week 3 Bleed and a dotted bar for Week 6 Bleed. Numerical values are provided above each bar.